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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s)	:	Hochstrasser et al.
Serial No.	:	10/695,194
For	:	DIAGNOSTIC METHOD FOR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES
Filed	:	October 28, 2003
Examiner	:	To be assigned
Art Unit	:	1645

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March 30, 2004
Carmella L. Stephens
Carmella L. Stephens (Reg. No. 41,328)

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SIR:

Submitted herewith are certified copies of Great Britain Patent Application Nos. GB 0121459.2, GB 0225245 and GB 0306290.8 to which priority was claimed upon the filing of the above-captioned application.

Applicants believe no fee is required. However, in the event a fee is required the Commissioner is hereby authorized to charge payment of any fee associated with this communication to Deposit Account No. 02-4377.

Respectfully submitted,

Dated: March 30, 2004

By: Carmella L. Stephens
Carmella L. Stephens
Reg. No. 41,328

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The Patent Office
Concept House
Cardiff Road
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South Wales
NP10 8QQ

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I also certify that the application is now proceeding in the name as identified herein.

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Dated 12 March 2004

GB 0121459.2

By virtue of a direction given under Section 32 of the Patents Act 1977, the application is proceeding in the name of

PROTEOME SCIENCES PLC,
Coveham House,
Downside Bridge Road,
COBHAM,
Surrey, KT11 3EP,
United Kingdom

Incorporated in the United Kingdom,

[ADP No. 07671670001]

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05SEP01 E657761-1 D01049
P01/7700 0.00-0121459.2

1. Your reference

ELEC,020-UK

2. Patent application number
(The Patent Office will fill in this part)

0121459.2

5 SEP 2001

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Universite de Geneve
Rue General-Dufour 24
Case Postale
CH-1211 Geneva 4
Switzerland

APPLICATION FILED 4/6/03
SECTION 32 (1977 ACT)

Patents ADP number *(if you know it)*

7153455002

If the applicant is a corporate body, give the country/state of its incorporation

Switzerland

4. Title of the invention

Diagnostic Method for Transmissible Spongiform Encephalopathies

5. Name of your agent *(if you have one)*

Brian Lucas
Lucas & Co.
135 Westhall Road
Warlingham
Surrey CR6 9HJ

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Patents ADP number *(if you know it)*

05815709001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
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See note (d))

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Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Brian Lucas Date 05.09.01

12. Name and daytime telephone number of person to contact in the United Kingdom

Brian Lucas - 01883 626211

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DIAGNOSTIC METHOD FOR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

BACKGROUND OF THE INVENTION

Field of the invention

This invention relates to a diagnostic method for a transmissible spongiform encephalopathy (TSE).

Description of the related art

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases of the central nervous system. They can be transmitted, inherited or occur sporadically and are observed in animals, e.g. as bovine spongiform encephalopathy (BSE) in cattle or scrapie in sheep, as well as in humans as Creutzfeldt-Jakob disease (CJD), Gerstman Sträussler Scheinker syndrome, Fatal Familial Insomnia or Kuru. They have a long incubation period, leading to ataxia, dementia, psychiatric disturbances and death. Neuropathological changes include vacuolar degeneration of brain tissue, astrogliosis and amyloid plaque formation. The diseases are difficult to diagnose pre-mortem.

The cerebrospinal fluid (CSF) of CJD patients displays two additional polypeptides (known as 14-3-3 polypeptides) by two-dimensional polyacrylamide gel electrophoresis [Harrington, M.G. New England Journal of Medicine *315*, 279 (1986), Hsich, G., Kenney, K., Gibbs, C.J., Lee, K.H. & Harrington, M. B. New England Journal of Medicine *335*, 924 (1996).] The function of these 14-3-3 polypeptides remains unclear in TSE. They can be used in a pre-mortem test for CJD diagnostic evaluation, but have low specificity.

Monoclonal antibodies to the abnormal form of prion protein (which is associated with CJD) are available and can be used in an enzyme-linked immunoassay, as described in PCT Specifications WO 98/23962 and 98/32710 and Schmerr, M.J., the Beckman Coulter Pace Setter Newsletter 3(2),1-4 (June 1999), but these procedures have not yet been fully developed.

PCT/EP 01/02894 relates to a diagnostic assay for TSEs in which the concentration of heart or brain fatty acid binding protein (H-FABP or B-FABP) is determined in a sample of body fluid.

US-A-6225047 describes the use of retentate chromatography to generate difference maps, and in particular a method of identifying analytes that are differentially present between two samples. One specific method described therein is laser desorption mass spectrometry.

WO 01/25791 describes a method for aiding a prostate cancer diagnosis, which comprises determining a test amount of a polypeptide marker, which is differentially present in samples of a prostate cancer patient and a subject who does not have prostate cancer. The marker may be determined using mass spectrometry, and preferably laser desorption mass spectrometry.

Development of new non-invasive TSE markers for body fluids (in particular, CJD and BSE markers in blood) and new methods of determining the markers would help clinicians to establish early diagnosis. This problem has now been solved by the present invention.

SUMMARY OF THE INVENTION

The present invention provides a method of diagnosis of a transmissible spongiform encephalopathy (TSE) or the possibility thereof in a subject suspected of suffering

from the TSE, which comprises subjecting a sample of body fluid taken from the subject to mass spectrometry, thereby to determine a test amount of a polypeptide in the sample, wherein the polypeptide is differentially contained in the body fluid of TSE-infected subjects and non-TSE-infected subjects, and has a molecular weight in the range of from 3500 to 30000; and determining whether the test amount is consistent with a diagnosis of TSE.

The invention also provides use of a polypeptide which is differentially contained in a body fluid of TSE-infected subjects and non-infected subjects, the polypeptide having a molecular weight in the range of from 3500 to 30000 and being determinable by mass spectrometry, for diagnostic, prognostic and therapeutic applications.

The invention further provides a kit for use in diagnosis of TSE, comprising a probe for receiving a sample of body fluid, and for placement in a mass spectrometer, thereby to determine a test amount of a polypeptide in the sample, wherein the polypeptide is differentially contained in the body fluid of TSE-infected subjects and non-TSE-infected subjects, and has a molecular weight in the range of from 3500 to 30000.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a spectral view of CSF from normal and CJD-infected samples using laser desorption/ionization mass spectrometry;

Figure 2 is a corresponding view highlighting a protein peak at about 4780 Da in CJD-infected CSF samples;

Figure 3 is a corresponding view highlighting protein peaks at about 6700 and 8600 Da in CJD-infected CSF samples;

Figure 4 is a corresponding view highlighting a protein peak at about 13375 Da in CJD-infected CSF samples;

Figure 5 is a spectral view of plasma from normal and BSE-infected samples using laser desorption/ionization mass spectrometry;

Figure 6 is a view corresponding to Figure 5 and highlighting a protein peak at about 10220 Da in BSE-infected plasma samples;

Figure 7 is a spectral view of plasma from CJD-infected patients (CJD+) and non-infected patients (CJD-) using laser desorption/ionization mass spectrometry; and

Figures 8A and 8B are views corresponding to Figure 7 and highlighting polypeptide peaks that are differentially expressed in the CJD+ and CJD- plasma samples.

DESCRIPTION OF PREFERRED EMBODIMENTS

The invention provides a method of diagnosis of a transmissible spongiform encephalopathy (TSE) or the possibility thereof in a subject suspected of suffering from the TSE. A sample of body fluid taken from the subject is subjected to mass spectrometry, to determine the presence or absence in the sample of a polypeptide marker which is differentially contained in the body fluid of TSE-infected subjects and non-infected subjects. The polypeptide marker has a molecular weight in the range of from 3500 to 30000, preferably from 3900 to 18000, and the presence or absence of the marker is indicative of TSE.

The method is applicable to all types of TSE, and to any human or animal suffering or suspected of suffering therefrom. The method is especially applicable to the diagnosis of CJD, especially new variant CJD, in human patients, and to BSE in ruminant animals such as cattle, and to BSE-like diseases in other animals, such as scrapie in sheep.

The term polypeptide includes proteins and protein fragments, as well as peptides modified by the addition of non-peptide residues, e.g. carbohydrates, phosphates, sulfates or any other post-translational modification.

The sample may be adsorbed on a probe under conditions which allow binding between the polypeptide and adsorbent material on the probe. The adsorbent material preferably comprises a metal chelating group complexed with a metal ion, and a preferred metal is copper. Prior to detecting the polypeptide, unbound or weakly bound materials on the probe may be removed with a washing solution, thereby enriching the polypeptide in the sample. The sample is preferably adsorbed on a probe having an immobilised metal affinity capture (IMAC) surface capable of binding the polypeptide. The sample may be also adsorbed on a probe having hydrophobic, strong anionic or weak cationic exchange surfaces under conditions which allow binding of the polypeptides. The probe may consist of a strip having several adsorbent wells, and be inserted into the spectrometer, then movable therein so that each well is in turn struck by the ionizing means (e.g. laser) to give a spectrometer reading. The polypeptide is preferably determined by surface-enhanced laser desorption/ionisation (SELDI) and time of flight mass spectrometry (TOF-MS).

In principle, any body fluid can be used to provide a sample for diagnosis, but preferably the body fluid is cerebrospinal fluid (CSF), plasma, serum, blood, urine or tears.

In one embodiment of the invention, the TSE is Creutzfeldt-Jakob disease (CJD). In this case, the polypeptide preferably has a molecular weight of about 4780, about 6700, about 8600 or about 13375, and the presence of one or more of such polypeptides is indicative of CJD. Alternatively, one or more polypeptides having a respective molecular weight of about 3970, about 3990, about 4294, about 4478, about 10075, about 11730, about 14043 or about 17839 is determined, and the absence of one or more of such polypeptides is indicative of CJD. As a further alternative, a polypeptide having a molecular weight of about 7770 is determined, and the presence of such polypeptide is indicative of CJD.

In another embodiment of the invention, the TSE is bovine spongiform encephalopathy (BSE). In this case, the polypeptide preferably has a molecular weight of about 10220, and the presence of the polypeptide is indicative of BSE.

In a further embodiment of the invention, the TSE is scrapie.

Measurement of the molecular weight of the polypeptide or polypeptides is effected in the mass spectrometer. The molecular weights quoted above can be measured with an accuracy of better than 1%, and preferably to within about 0.1%. The term "about" in connection with molecular weights therefore means within a variation of about 1%, preferably within about 0.1%, above or below the quoted value.

The invention also relates to the use of a polypeptide which is differentially contained in a body fluid of TSE-infected subjects and non-infected subjects, the polypeptide having a molecular weight in the range of from 3500 to 30000 and being determinable by mass spectrometry, for diagnostic, prognostic and therapeutic applications. This may involve the preparation and/or use of a material which recognizes, binds to or has some affinity to the above-mentioned polypeptide. Examples of such materials are antibodies and antibody chips. The term "antibody" as used herein includes polyclonal antiserum, monoclonal antibodies, fragments of antibodies such as Fab, and genetically engineered antibodies. The antibodies may be chimeric or of a single species. The above reference to "prognostic" applications includes making a determination of the likely course of a TSE by, for example, measuring the amount of the above-mentioned polypeptide in a sample of body fluid. The above reference to "therapeutic" applications includes, for example, preparing materials which recognize, bind to or have affinity to the above-mentioned polypeptides, and using such materials in therapy. The materials may in this case be modified, for example by combining an antibody with a drug, thereby to target the drug to a specific region of the animal to be treated.

The methodology of this invention can be applied to the diagnosis of any TSE. Body fluid samples are prepared from infected and non-infected subjects. The samples are applied to a probe having a surface treated with a variety of adsorbent media, for

differential retention of peptides in the sample, optionally using washing liquids to remove unbound or weakly bound materials. If appropriate, energy-absorbing material can also be applied. The probe is then inserted into a mass spectrometer, and readings are taken for the various sample/adsorbent combinations using a variety of spectrometer settings. Comparison of the infected and non-infected samples under a given set of conditions reveals one or more polypeptides which are differentially expressed in the infected and non-infected samples. The presence or absence of these polypeptides can then be used in the testing of a fluid sample from a subject under the same conditions (adsorbent, spectrometer settings etc.) to determine whether or not the subject is infected.

The above reference to "presence or absence" of a polypeptide should be understood to mean simply that there is a significant difference in the amount of a polypeptide which is detected in the infected and non-infected sample. Thus, the "absence" of a polypeptide in a test sample may include the possibility that the polypeptide is actually present, but in a significantly lower amount than in a comparative test sample. According to the invention, a diagnosis can be made on the basis of the presence or absence of a polypeptide, and this includes the presence of a polypeptide in a significantly lower or significantly higher amount with reference to a comparative test sample.

The following Examples illustrate the invention.

EXAMPLE 1

The objective of the present study was to detect specific polypeptides in body fluids (cerebrospinal fluid, plasma and others) of Creutzfeld-Jacob affected patients. Samples were analysed by the Surface Enhanced Laser Desorption Ionization (SELDI) Mass Spectroscopy (MS) technology. This technology encompasses micro-scale affinity capture of proteins by using different types of retentate chromatography and then analysis by time of flight mass spectrometry. Different maps are thus generated each corresponding to a typical protein profiling of given samples that were analysed with a Ciphergen Biosystem PBS II mass spectrometer (Freemont, CA, USA).

Differential expressed peaks were identified when comparing spectra generated in a group of cerebrospinal fluid (CSF) samples from CJD-affected patients with a group of dementia-affected patients.

The SELDI analysis was performed using 2µl of crude human CSF samples in order to detect specific polypeptides with metal affinity. An immobilized copper affinity array (IMAC-Cu^{++}) was employed in this approach to capture proteins with affinity for copper to select for a specific subset of proteins from the samples. Captured proteins were directly detected using the PBSII Protein Chip Array reader (Ciphergen Biosystems, Freemont, CA, USA).

The following protocol was used for the processing and analysis of ProteinChip arrays using Chromatographic TED-Cu(II) adsorbent array. TED is a (tris(carboxymethyl)ethylenediamine-Cu) adsorbent coated on a silicon oxide-coated stainless steel substrate.

- The surface was first loaded with 10 µl of 100 mM copper sulfate to each spot and incubated for 15 minutes in a wet chamber.
- The chip was thereafter washed by two quick rinses with deionized water for about 10 seconds to remove the excess unbound copper.
- Before loading the samples, the I-MAC 3 array was equilibrated once with 5 µl of PBS NaCl 0.5 M for 5 minutes.
- After removing the equilibration buffer, 3 µl of the same buffer were added before applying 2 µl of CSF. The chip was incubated for 20 minutes in a wet chamber.
- The samples were thereafter removed and the surface was washed three times with the equilibration buffer (5 minutes each).
- Two quick final rinses with water were performed.
- The surface was allowed to air dry, followed by the addition of 0.5 µl of saturated sinapinic acid (SPA, Ciphergen Biosystem) prepared in 50% acetonitrile, 0.5% trifluoroacetic acid.
- The chip was air dried again before analysis of the retained protein on each spot with laser desorption/ionization time-of-flight mass spectrometry.

- The protein chip array was inserted into the instrument and analysed once the appropriate detector sensitivity and laser energy have been established to automate the data collection.
- The obtained spectra were analysed with the Biomark Wizard software (Ciphergen Biosystems, Freemont, CA, USA) running on a Dell Dimension 4100 PC. It generates consistent peak sets across multiple spectra.

Figures 1 to 4 shows the results of a comparative study which has been undertaken between CSF from CJD diagnosed patients and normal CSF, using the IMAC 3 protein chip array prepared as described above. In this study, we found that four peaks were significantly differentially increased in CSF from CJD affected patients. Their molecular weights are respectively about 4780, 6700, 8600 and 13375 (mass accuracy is around 0.1%). Figure 1 shows two spectral views, respectively of the normal and CJD sample, from 0 to 100,000 Da. Figure 2 shows the protein peak of 4780 Da, Figure 3 shows the protein peaks of 6700 and 8600 Da, and Figure 4 shows the protein peak of 13375 Da. These data demonstrate that the peaks of about 4780, 6700, 8600 and 13375 Da can be used to diagnose CJD in CSF samples.

EXAMPLE 2

Example 1 was repeated using plasma samples from BSE-infected cattle (BSE +) and non-infected cattle (BSE -). The results are shown in Figures 5 and 6. Figure 5 shows a spectral view of each kind of sample from 0 to 50,000 Da. We observed that a protein around 10220 Da was significantly increased in BSE + plasma samples, as illustrated in Figure 6. This demonstrates that the peak of about 10220 Da can be used to diagnose BSE in plasma samples.

EXAMPLE 3

Example 2 was repeated using plasma samples from CJD-infected patients (CJD +) and non-infected patients (CJD-, also referred to as CTS = Swiss Transfusion Centre). The results are shown in Figures 7 and 8. Figure 7 shows a spectral view of each kind of sample from 0 to 50,000 Da. We observed that polypeptides of about 3970, about

3990, about 4294, about 4478, about 10075, about 11730, about 14043 or about 17839 were significantly decreased in CJD + plasma samples, as illustrated in Figures 8A and B. We also observed that a peak of about 7770 Da was increased in CJD + plasma samples, as illustrated in Figure 8B. This demonstrates that the peak of about 3970, about 3990, about 4294, about 4478, about 10075, about 11730, about 14043, about 17839 or about 7770 Da can be used to diagnose CJD in plasma samples.

* * * * *

Each of the above cited publications is herein incorporated by reference to the extent to which it is relied on herein.

7. A method according to any of Claims 1 to 6, in which the body fluid is cerebrospinal fluid, plasma, serum, blood or tears.

8. A method according to any of Claims 1 to 7, in which a plurality of peptides is determined in the sample.

9. A method according to any of Claims 1 to 8, in which the TSE is Creutzfeldt-Jakob disease (CJD).

10. A method according to Claim 9, in which one or more polypeptides having a respective molecular weight of about 4780, about 6700, about 8600 or about 13375 is determined, and the presence of one or more of such polypeptides is indicative of CJD.

11. A method according to Claim 9 or 10, in which one or more polypeptides having a respective molecular weight of about 3970, about 3990, about 4294, about 4478, about 10075, about 11730, about 14043 or about 17839 is determined, and the absence of one or more of such polypeptides is indicative of CJD.

12. A method according to any of Claims 9 to 11, in which a polypeptide having a molecular weight of about 7770 is determined, and the presence of such polypeptide is indicative of CJD.

13. A method according to any of Claims 1 to 8, in which the TSE is bovine spongiform encephalopathy (BSE).

14. A method according to Claim 13, in which the polypeptide has a molecular weight of about 10220, and the presence of the polypeptide is indicative of BSE.

15. A method according to any of Claims 1 to 8, in which the TSE is scrapie.

16. Use of a polypeptide which is differentially contained in a body fluid of TSE-infected subjects and non-infected subjects, the polypeptide having a molecular

CLAIMS

1. A method of diagnosis of a transmissible spongiform encephalopathy (TSE) or the possibility thereof in a subject suspected of suffering from the TSE, which comprises subjecting a sample of body fluid taken from the subject to mass spectrometry, thereby to determine a test amount of a polypeptide in the sample, wherein the polypeptide is differentially contained in the body fluid of TSE-infected subjects and non-TSE-infected subjects, and has a molecular weight in the range of from 3500 to 30000; and determining whether the test amount is consistent with a diagnosis of TSE.

2. A method according to Claim 1, in which the polypeptide is present in the body fluid of TSE-infected subjects and not present in the body fluid of non-TSE-infected subjects, whereby the presence of the polypeptide in a body fluid sample is indicative of TSE.

3. A method according to Claim 1, in which the polypeptide is not present in the body fluid of TSE-infected subjects and present in the body fluid of non-TSE-infected subjects, whereby the non-presence of the polypeptide in a body fluid sample is indicative of TSE.

4. A method according to any of Claims 1 to 3, in which the mass spectrometry is laser desorption/ionization mass spectrometry.

5. A method according to any of Claims 1 to 4, in which the sample is adsorbed on a probe having an immobilised metal affinity capture (IMAC), hydrophobic, strong anionic or weak cationic exchange surface capable of binding the polypeptide.

6. A method according to any of Claims 1 to 5, in which the polypeptide is determined by surface-enhanced laser desorption/ionisation (SELDI) and time of flight mass spectrometry (TOF-MS).

weight in the range of from 3500 to 30000 and being determinable by mass spectrometry, for diagnostic, prognostic and therapeutic applications.

17. Use for diagnostic, prognostic and therapeutic applications of a material which recognizes, binds to or has affinity for a polypeptide which is differentially contained in a body fluid of TSE-infected subjects and non-infected subjects, the polypeptide having a molecular weight in the range of from 3500 to 30000 and being determinable by mass spectrometry.

18. Use according to Claim 17, in which the material is an antibody or antibody chip.

19. A kit for use in diagnosis of TSE, comprising a probe for receiving a sample of body fluid, and for placement in a mass spectrometer, thereby to determine a test amount of a polypeptide in the sample, wherein the polypeptide is differentially contained in the body fluid of TSE-infected subjects and non-TSE-infected subjects, and has a molecular weight in the range of from 3500 to 30000.

20. A kit according to Claim 19, in which the probe contains an adsorbent for adsorption of the polypeptide.

21. A kit according to Claim 20, further comprising a washing solution for removal of unbound or weakly bound materials from the probe.

ABSTRACT

DIAGNOSTIC METHOD FOR TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

Transmissible spongiform encephalopathy (TSE) is diagnosed in a subject by using mass spectrometry to observe a polypeptide in a sample of body fluid taken from the subject. The polypeptide is differentially contained in the body fluid of TSE-infected subjects and non-infected subjects, and has a molecular weight in the range of from 3500 to 30000.

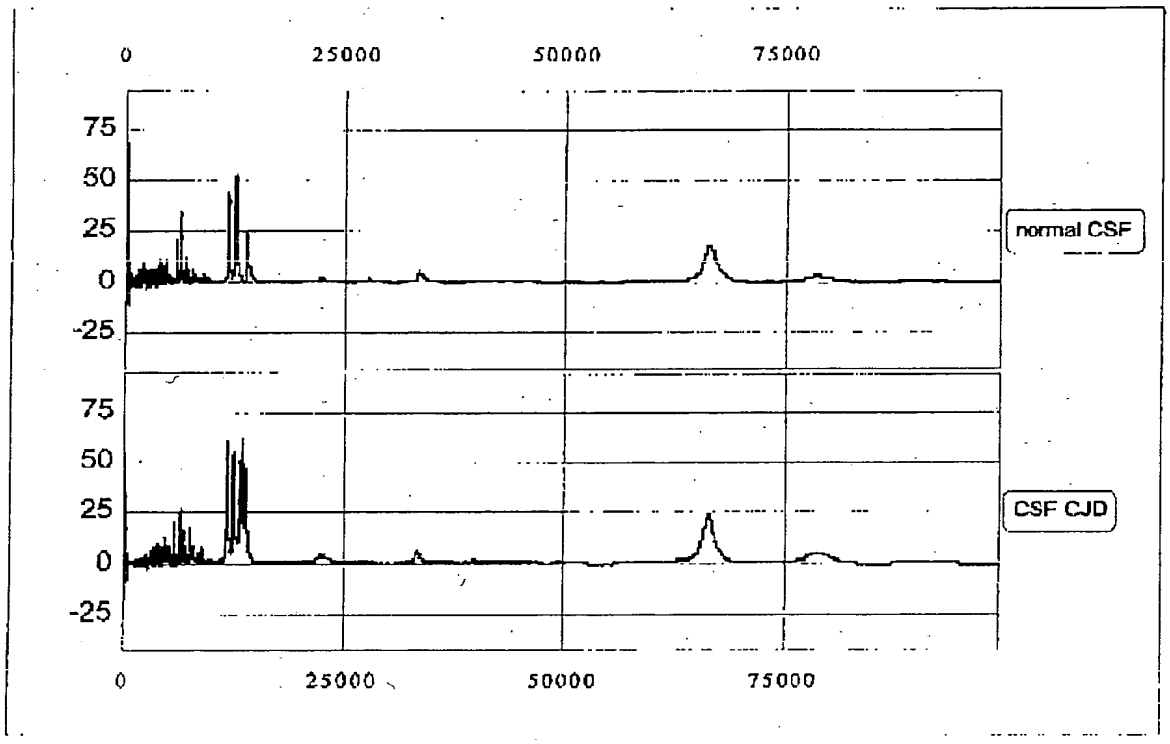
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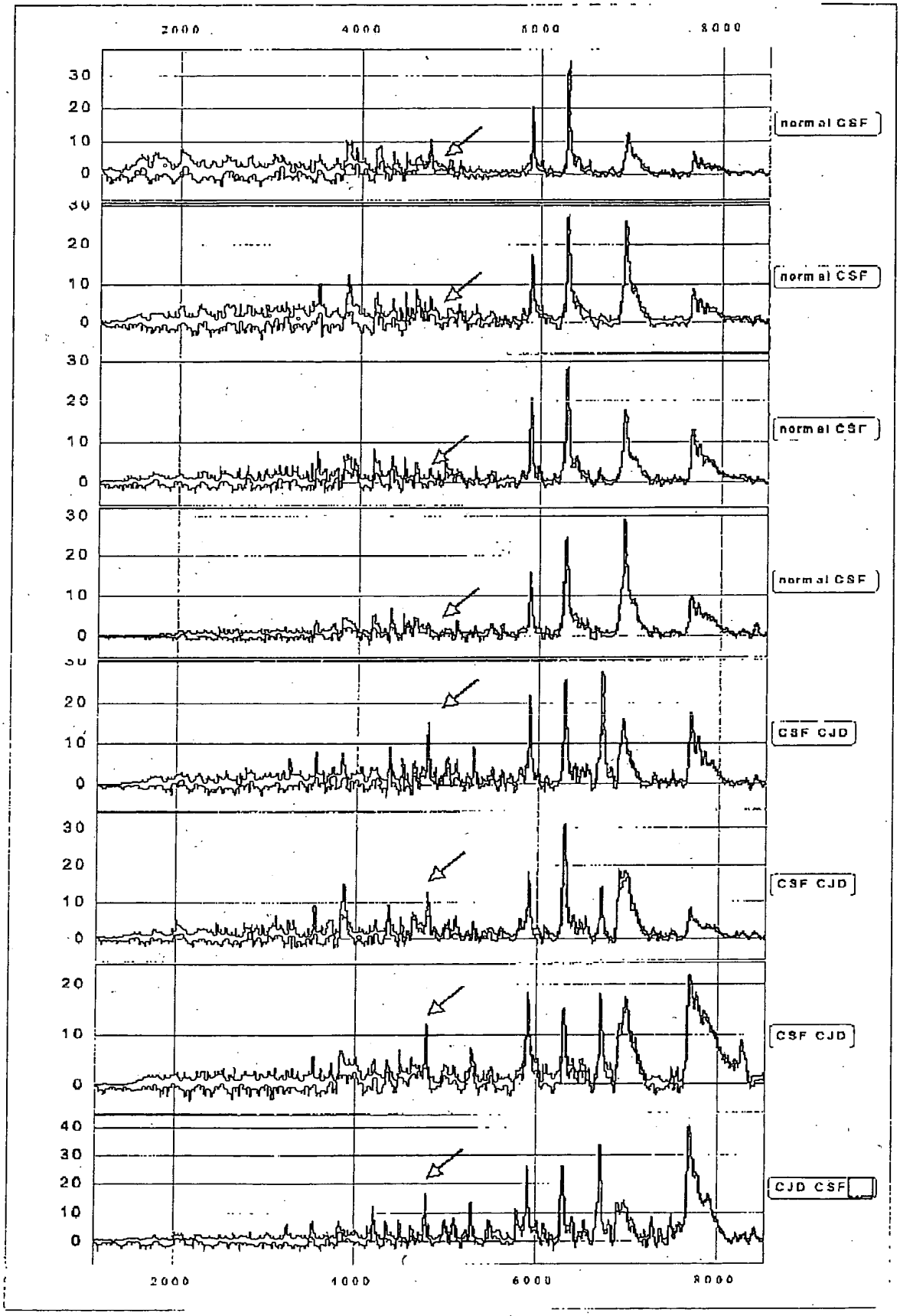
Figure 1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normal CSF
normal CSF
CSF CJD
CSF CJD
CSF CJD
CJD CSF

Figure 2

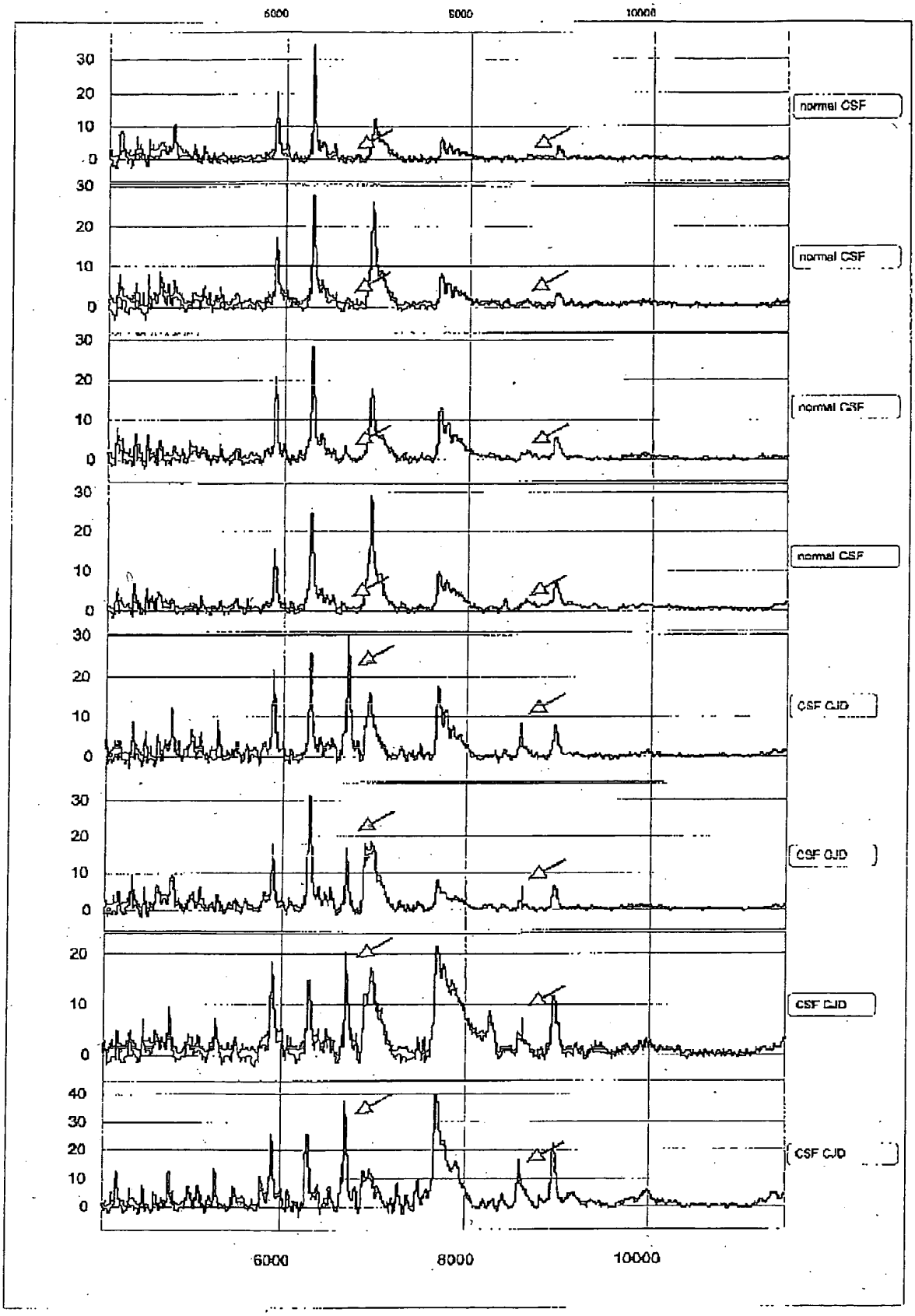
6000
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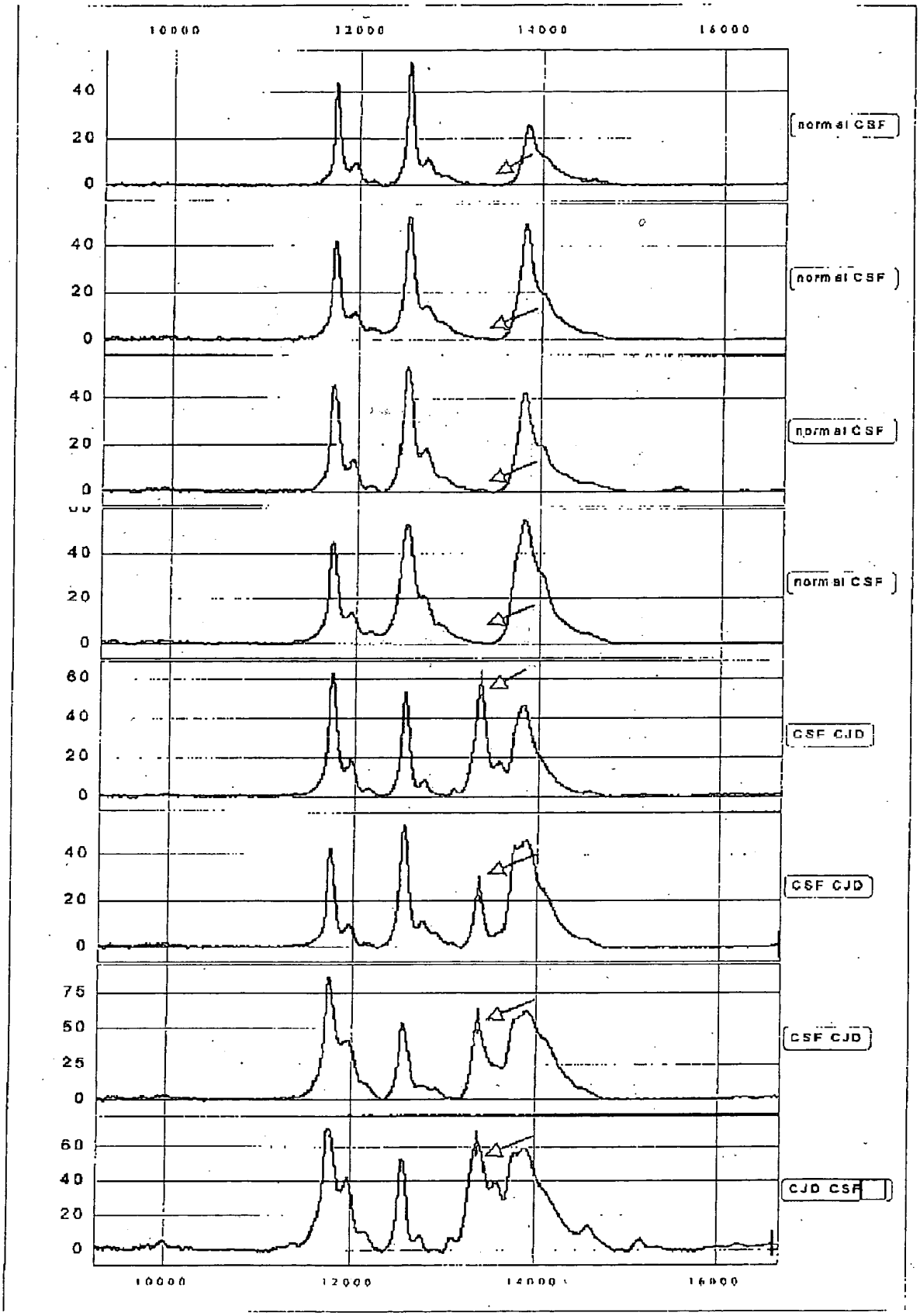
Figure 3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16000
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normal CSF
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CSF CJD
CSF CJD
CSF CJD
CJD CSF

Figure 4

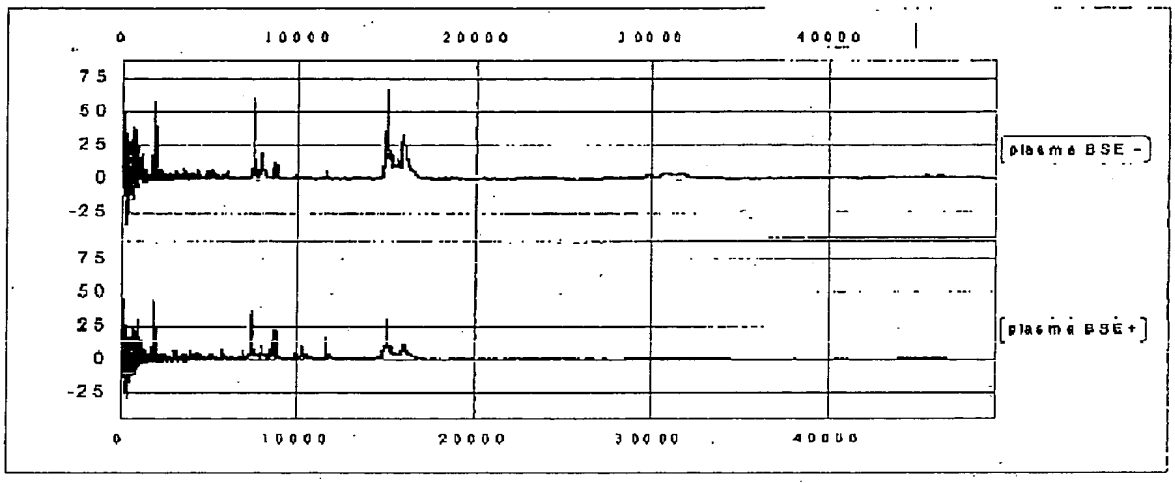

Figure 5

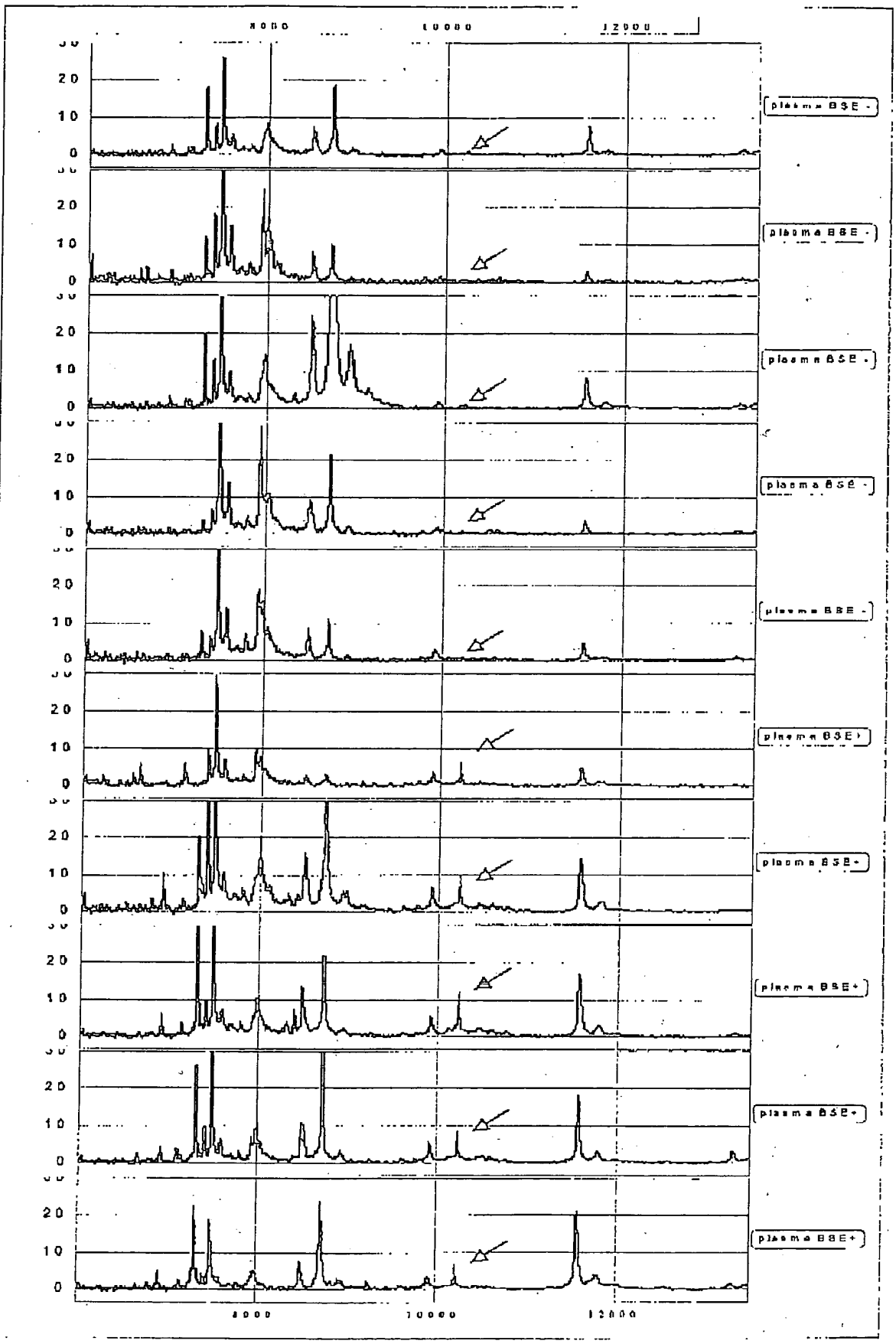
8000
10000
12000
20
10
0
plasma BSE -
plasma BSE -
plasma BSE -
plasma BSE -
plasma BSE -
plasma BSE+
plasma BSE+
plasma BSE+
plasma BSE+
8000
10000
12000

Figure 6

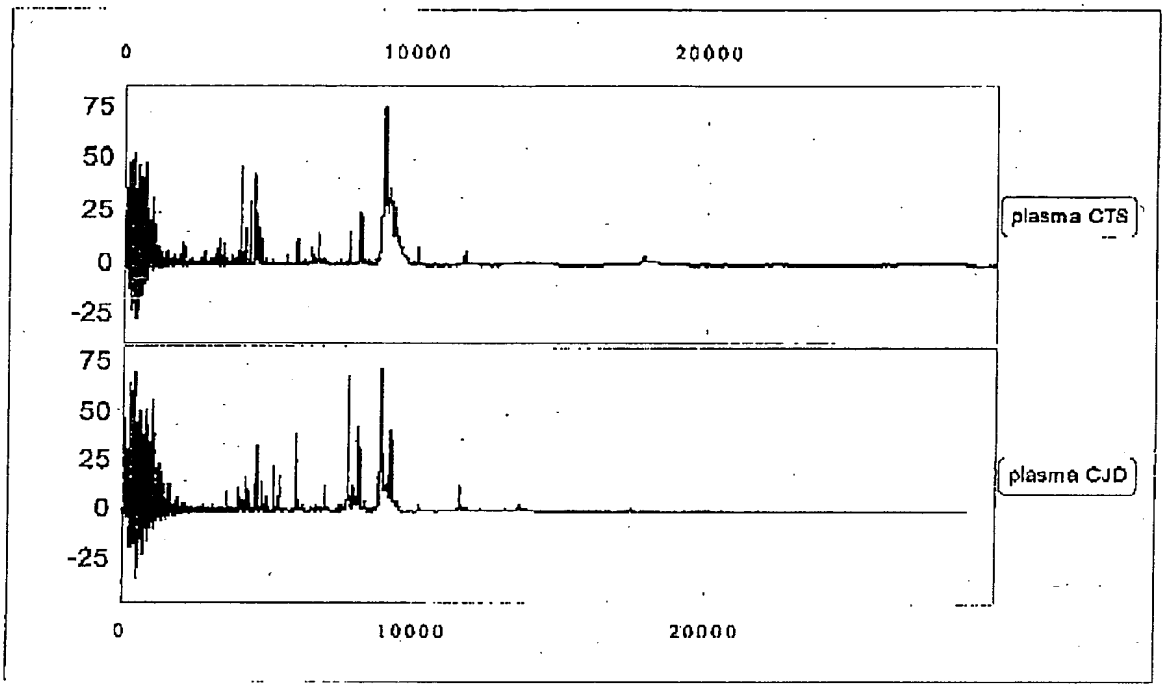
0
10000
20000
75
50
25
0
-25
plasma CTS
75
50
25
0
-25
plasma CJD
0
10000
20000

Figure 7

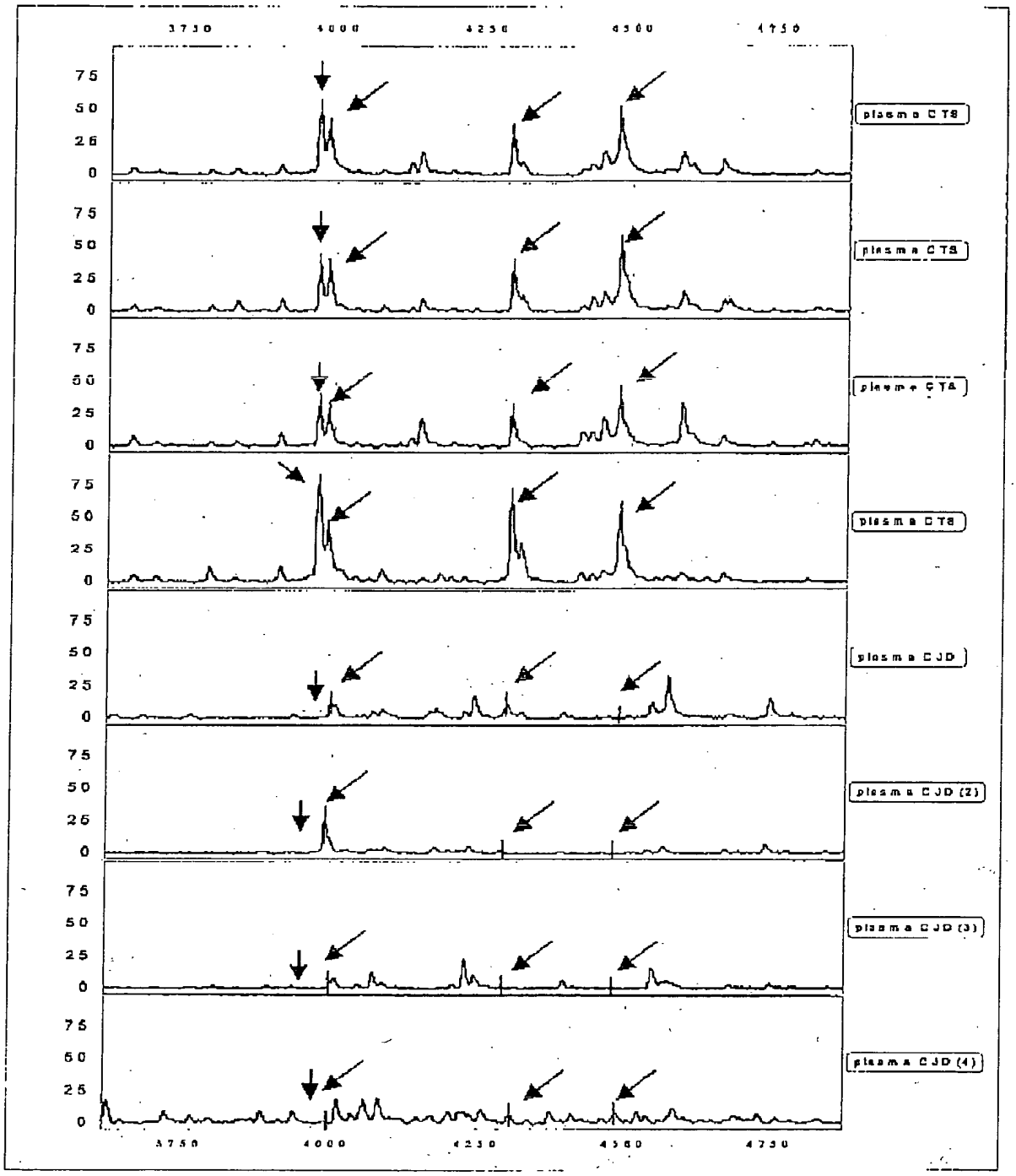

Figure 8A : 3970, 3990, 4294, 4478

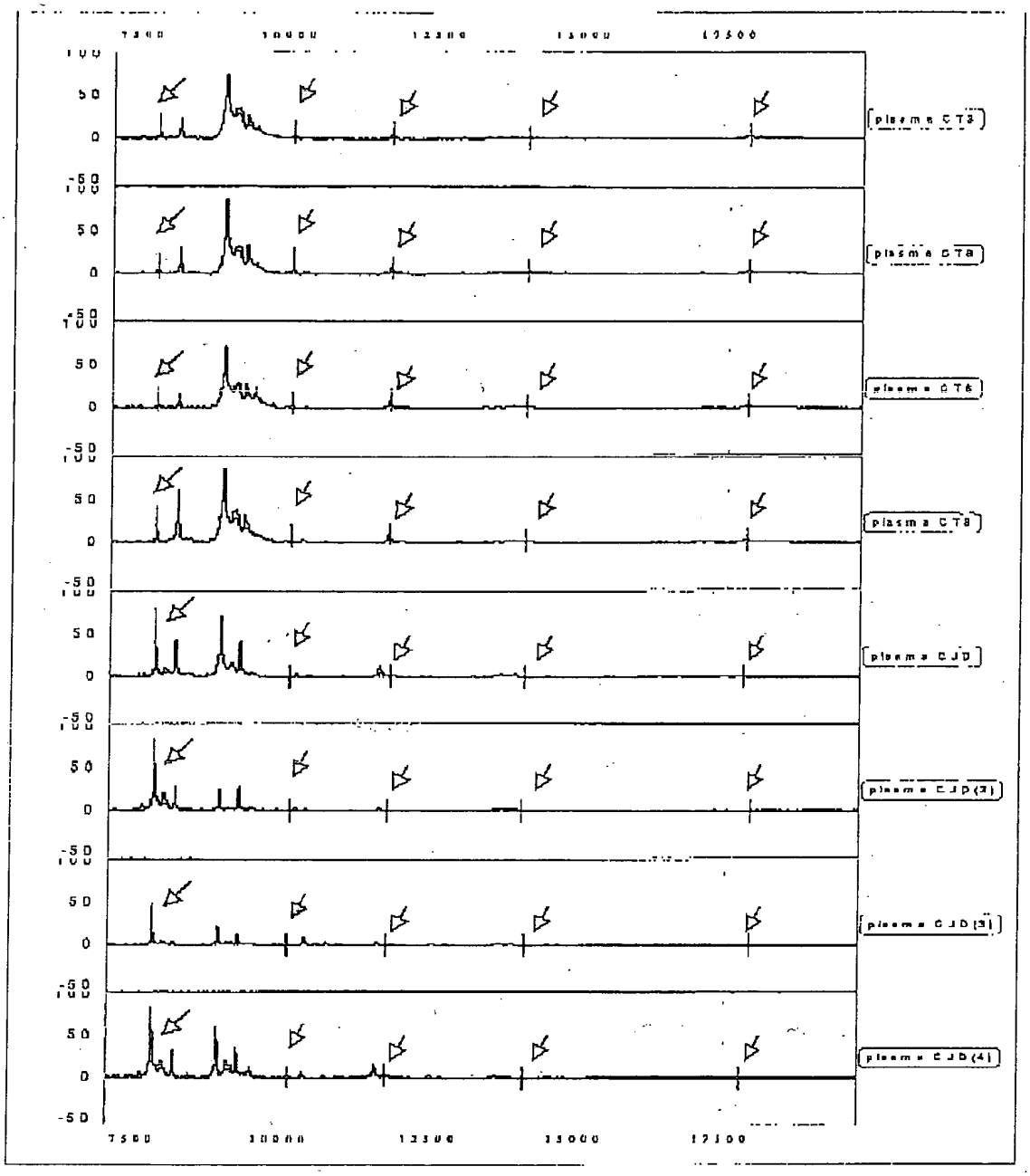

Figure 8B : 7770, 10075, 11730,14043, 17839